Tridocosahexaenoina-AOX®

BRUDYLAB SCIENCE FUNDAMENTALS 2000-2018

BRUDYLAB® I MEDICAL NUTRITION SPECIALISTS

The European Office of Patents has recently recognized the success of Brudy's science by the concession, between others, of the **patent EP1962825**. This patent has not only exceeded the most exigent novelty exams and inventive activity of this office, but also confirming its validity and efficacy by firm decision given on May 12, 2017, and with rejection of all oppositions posed by various DHA producing companies. With the trade name of **ALGATRIUM®**, Brudylab produces and markets the **only available products containing highly concentrated DHA-triglyceride (Tridocosahexaenoina-AOX®) authorized for antioxidative protection in eye diseases associated to oxidative stress.** In this monograph, we present the science fundamentals giving support to the antioxidant-anti-inflammatory activity of our molecule, Tridocosahexaenoina-AOX®, which has been patented as a cell antioxidant; it induces intracellular glutathione synthesis. We explain the synthesis method, our strict control of environmental pollutants, the importance of DHA for the visual and intelectual human development, and the beneficial healthy claims approved by the European Commission. We explain our *"in Vitro"* tests, of induced oxidative stress on human cell cultures, the dose-response bioavailability trials, as also our main clinicals trials done to demonstrate the antioxidant and anti-inflammatory efficacy at a clinical level. We also show a summary of all our clinical experience. Our achievements after 18 years of research efforts are the result of a private company working in close relation with the University for the benefit of the human health.

BRUDYLAB, SL.

Abreviations

ADHD: Attention Deficit Hyperactivity Disorder
ALA: Alphalinolenic acid
ARA: Arachidonic acid
ARPE-19: Inmortalized human pigmentary epithelium cells from the retina
AOX: Antioxidant
CG: Control Group
DE: Dry Eye
DHA: Docosahexaenoic acid
DNA: Desoxiribonucleic acid
EPA: Eicosapentaenoic acid
ETDRS: Early Treatment Diabetic Retinopathy Study
GPAA: Primary Open Angle Glucoma
GPX: Glutathine Peroxidase
GRed: Glutathione Reductase
GSH: Reduced Glutathione
GSSG: Oxidized Glutathione
HIV: Human Immunodeficient Virus
IL: Interleukines
IMA: Ischemic Modifyed Albumin
IOP: Intraocular Pressure
MDA: Malondialdehyde
MGD: Meibomian Gland Disfunction
MUFA: Monounsaturated fatty acids
NF-κβ: Nuclear Factor κβ
OCT: Optical Coherence Tomography
OMPD: Ocular Macular Pigment Density
OSDI: Ocular Surface Disease Index
POAG: Primary Open Angle Glaucoma
PUFA: Polyunsaturated fatty acids
ROX: Oxigenated free-radicals
SFA: Saturated fatty acids
Sn-1,2,3: Stereospecific number-1,2,3
SOD: Superoxide dismutase
TBUT: Tear Break-Up Time
TG: Triglyceride
TNF-α: Tumor Necrosis Factor-α
TUNEL: Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling
VA: Visual Acuity

* Tridocosahexaenoina-AOX®: Antioxidant DHA-Triglyceride. It is Brudy's patented DHA-Triglyceride

BRUDYLAB, SL Medical Department
Biochemistry and Molecular Biology Department, Biology Faculty, University of Barcelona

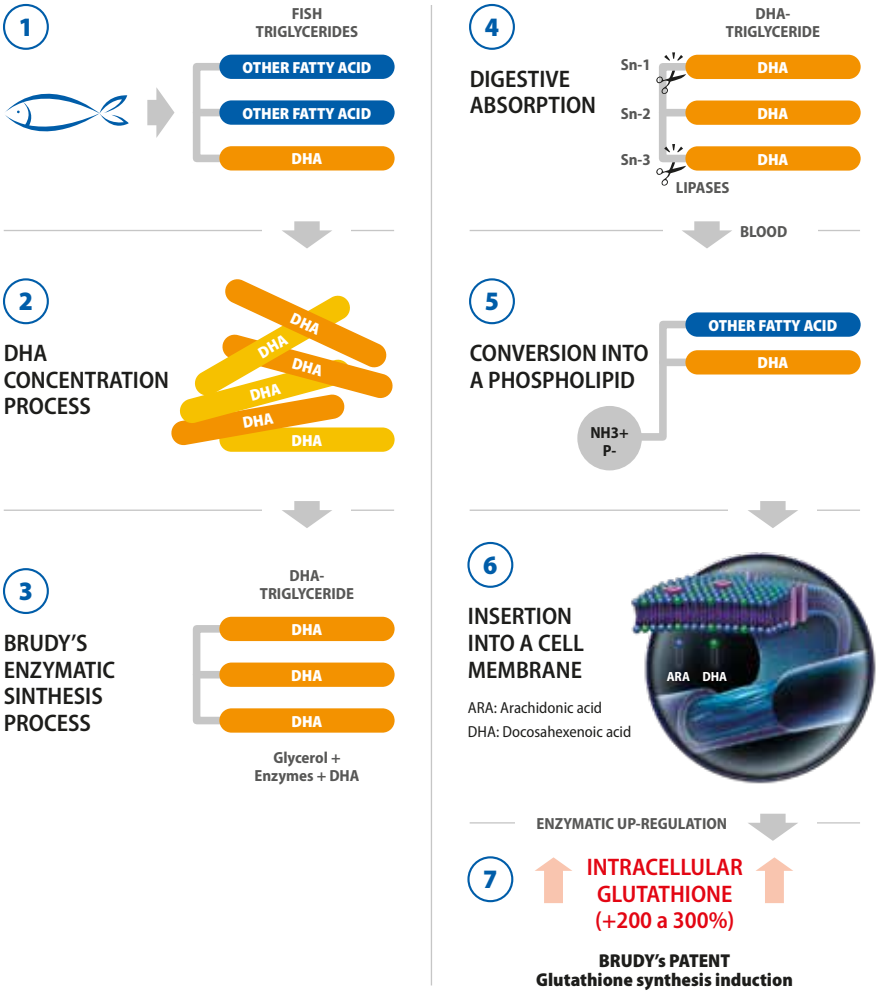
TRIDOCOSAHEXAENOINA-AOX® SYNTHESIS

What is basic is to know about the DHA human physiology

Tridocosahexaenoina-AOX® synthesis process

Conversion of fish-triglycerides into human-like triglycerides, as they are found in human maternal milk, having DHA in central position(Sn-2).

1. Departing from fish (Tuna fish and anchovies from Peru) triglycerides.
2. Cholesterol, Phytanic acid and all the fatty acids but DHA are eliminated. It is obtained a 70% concentrated DHA-ethyl ester.
3. After total extraction of ethanol, new triglycerides are enzymatically re-synthetized, having DHA in central position Sn-2 around 80% of the molecules.
4. Digestive lipases can break bonds in Sn-1 and Sn-3 positions of the triglycerides. A monoglyceride having DHA in central position is absorbed.
5. When the liver detects PUFA in central position (Sn-2), they are immediately transformed into DHA-phospholipids.
6. DHA-phospholipids are inserted into a cell membrane bilayer.
7. The larger DHA presence in the cell membrane is inducing glutathione synthesis (GSH).



We demand more than to meet the standards of environmental pollutants control, far below the allowed maximum levels[1]

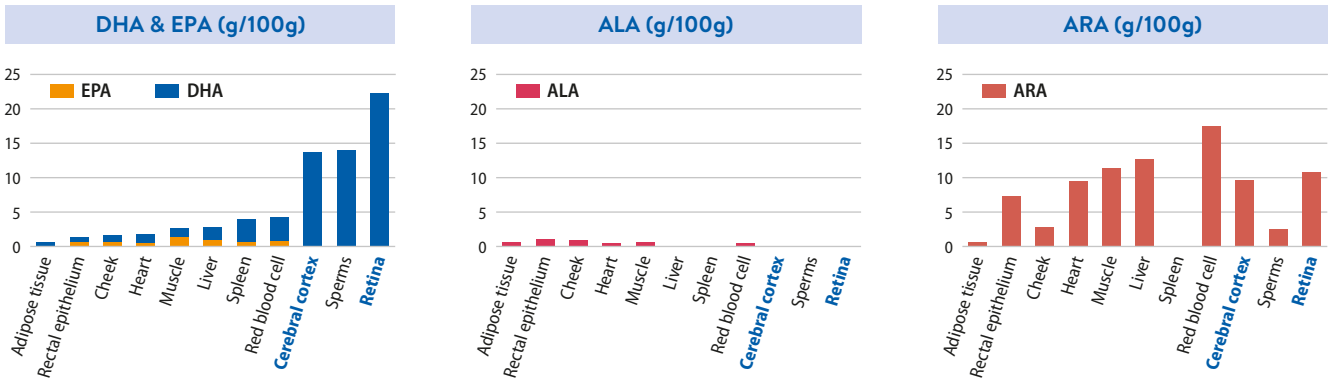
POLLUTANTS	Allowed maximum levels		Brudy batches
	European legislation	GOED	Results
Pb	0,1 ppm	0,1 ppm	< 0,02 ppm
As	-	0,1 ppm	< 0,05 ppm
Hg	0,5 ppm	0,1 ppm	< 0,005 ppm
Cd	0,1 ppm	0,1 ppm	< 0,005 ppm
Sn	-		< 0,2 ppm
Cu	-		< 0,1 ppm
Fe	-		< 0,1 ppm
Dioxins	1,75 pg/g		< 0,4 pg/g
Dioxins and similar PBC	6 pg/g		< 3,0 pg/g
PBCs	200 ng/g		< 15 ng/g
PAH (Benzopirenes)	2,0ppb		<2,0 ppb

PBCs: Polychlorinated biphenyls / GOED: Global Organization for EPA and DHA / PAH: Polycyclic Aromatic Hydrocarbons / ppm: Parts per million

DHA HUMAN NEEDS ARE A MUST

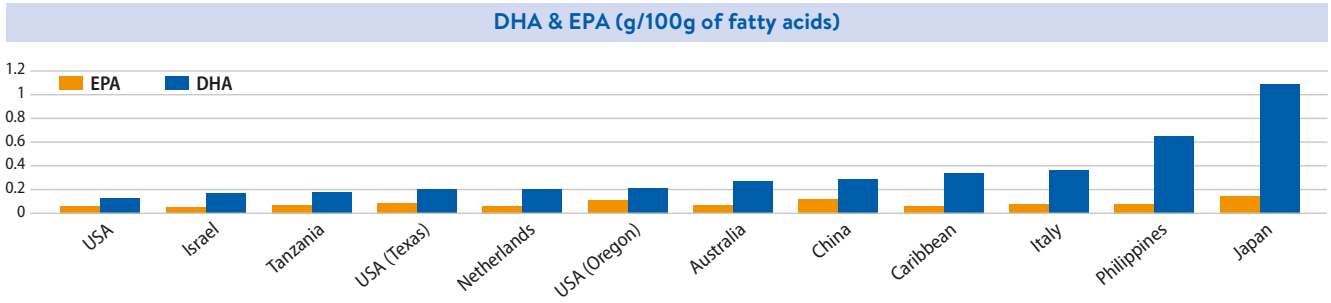
A basic need for intellectual and visual development in humans[2]

Concentration of fatty acids found in human tissues (g/100g of total fatty acids)[2]
of USA, Canada, Australia and European adults



EPA: Eicosapentaenoic acid / DHA: Docosahexaenoic acid / ALA: Alphalinolenic acid / ARA: Arachidonic acid

DHA & EPA content in human mothers milk from a diverse origin[2]
depending on their dietetic culture



DHA Healthy Claims approved by European Comission

DHA HEALTHY CLAIMS APPROVED BY EUROPEAN COMISSION	CONSUMER SHOULD BE INFORMED THAT THE BENEFIT IS OBTAINED WITH INGESTION OF:
DHA (and also DHA+EPA) contribute to the normal function of the heart.	DHA 250mg/day (EU 432/2012) for adults. EFSA journal 2014; 12(10): 3840 for child of 2 to 18 years.
DHA contributes to maintenance of normal vision.	
DHA contributes to maintenance of normal brain function.	
DHA intake contributes to normal visual development of infants up to 12 months of age.	DHA 100mg/day (EU 440/2011).
DHA maternal intake contributes to the normal development of the eye of the fetus and breastfed infants.	(For pregnant and lactating women) DHA 200mg/day additional to 250mg/day recommended for adults (EU 440/2011).
DHA maternal intake contributes to the normal brain development of the fetus and breastfed infants.	
DHA (and also for combined DHA+EPA) contribute to the maintenance of normal blood triglyceride levels.	DHA 2g/day, also for DHA+EPA. Not to exceed a supplemental daily intake of 5g/day (EU 536/2013).
DHA and EPA contribute to the maintenance of normal blood pressure.	DHA 3g/day, also for combined DHA+EPA. Not to exceed a supplemental daily intake of 5g/day (EU 536/2013).

Consultation of European regulations should be made in: **www.eur-lex.europa.eu** (searches must be done with the year and the document number that appear in the right column of the table)

OUR PATENT

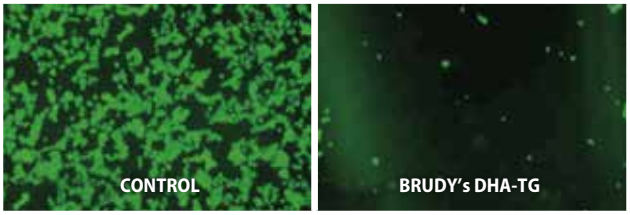
Tridocosahexaenoina-AOX® cell antioxidant effect[3]

When a cell membrane is highly enriched with DHA, being DHA easily oxidized, the cell makes a genic activation of intracellular glutathione (GSH) synthesis, by means of an enzymatic up-regulation. GSH concentration is increased between 200 to 300% inside the cell cytoplasm.

GSH is the main antioxidant molecule, electron donor, in mammal cells; scavenger of oxygen free-radicals (ROX) protecting DNA, lipids and proteins from the cell organelles of being oxidized.

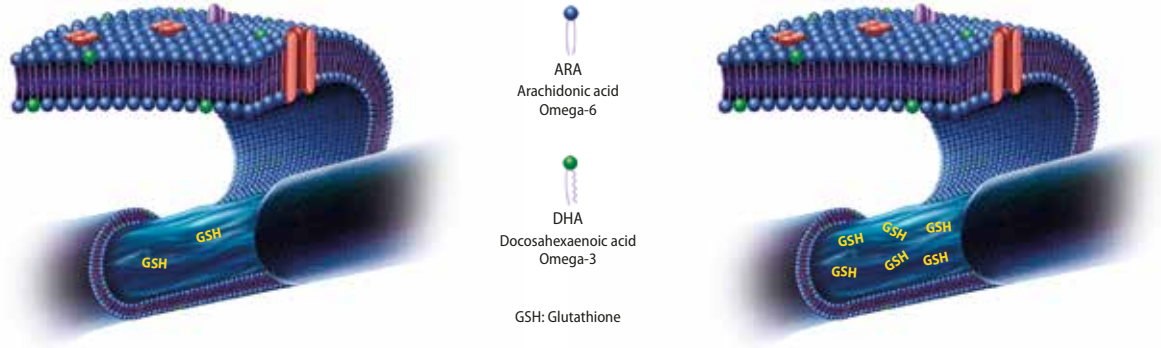
FIBROBLASTS

RETINA ARPE-19 CELLS



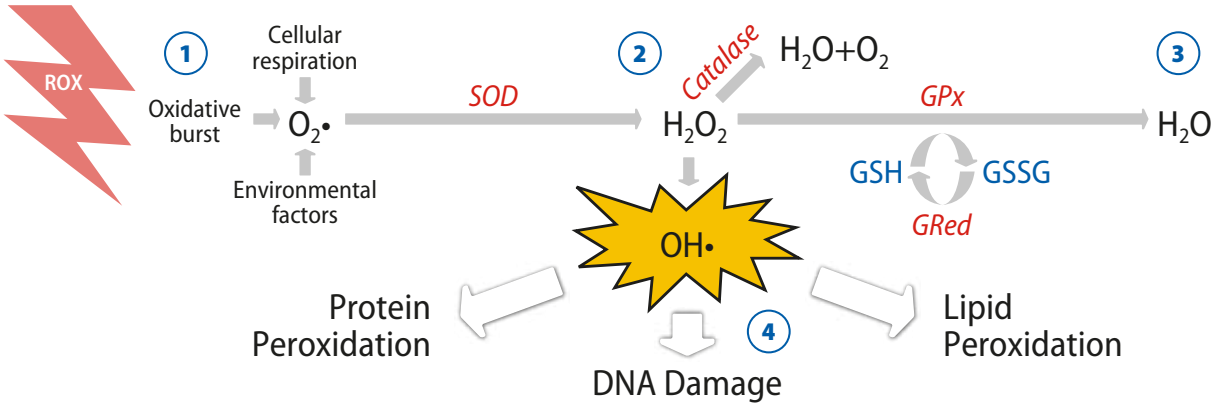
Induced ROX are reduced by 50% in human cell cultures when they are pre-incubated in Tridocosahexaenoina-AOX®.

SECTION OF A NEURON MEMBRANE



Cells get shielded against any internal or external oxidation excess based on a their larger glutathione production. Glutathione is responsible of scavenging oxygen free radicals as an electron donor.

CELLULAR ENZIMATIC MECHANISMS BY WHICH OXIGENATED FREE RADICALS ARE SCAVENGED



Anion superoxide (O_2•) is converted into hydrogen peroxide (H_2O_2) by superoxide dismutase to avoid formation of the hydroxil radical (OH•). Catalase and glutathione peroxidase are converting the peroxide into molecular oxygen and water (H_20+O_2) by using the electrons given by GSH, thus avoiding oxidative harm on to the DNA, lipids and proteins of the cell.

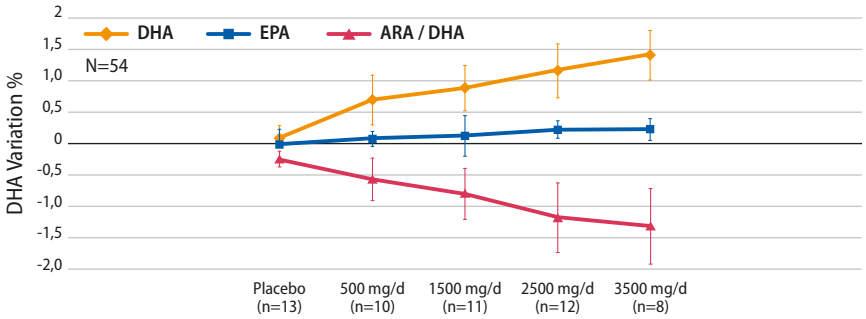
TRIDOCOSAHEXAENOINA-AOX® DIGESTIVE ABSORPTION

Bioavailavility: dose-response trials

Short term[4]

DHA concentration in erythrocyte membrane, after supplementing 5 groups of healthy volunteers with variable doses of Tridocosahexaenoina-AOX® or with Placebo, is dose dependent.

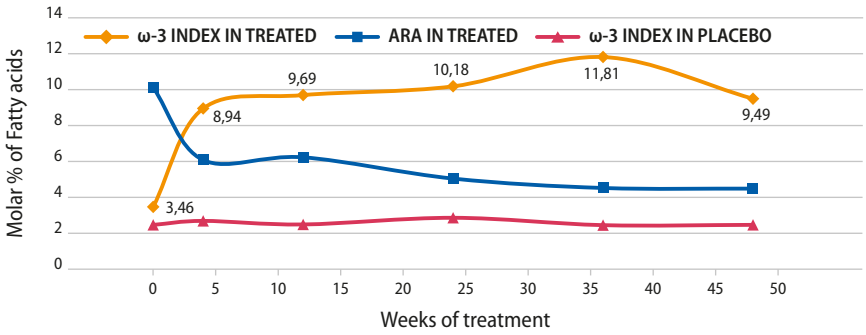
MARKER: ERYTHROCYTE MEMBRANE



Long term[5]

A controlled, randomized, double-blinded clinical trial including a sample of 84 HIV+ patients, being supplemented either with Tridocosahexaenoina-AOX® 4g/day or Placebo during 1 year. PUFA concentration is detected by means of gas Chromatography. Only the active supplementation is significantly increasing DHA concentration in the erythrocyte cell membrane, and reducing the ARA levels.

MARKER: ERYTHROCYTE MEMBRANE

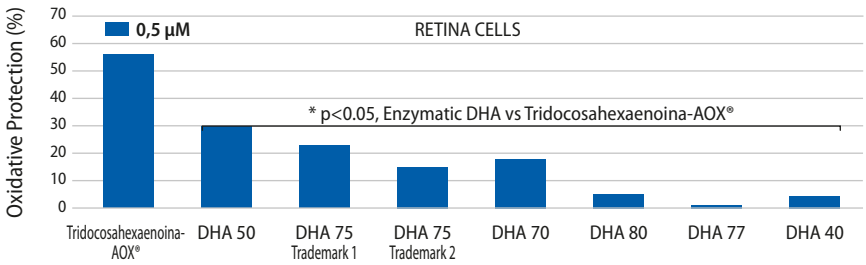


THE ANTIOXIDANT EFFICACY IS NOT RELATED WITH THE DHA CONCENTRATION IN THE TRIGLYCERIDE

The antioxidant efficacy is related with the DHA 6 double bonds integrity, but also with DHA occupying the central position in the triglyceride

Tridocosahexaenoina-AOX® (ENZYMATIC DHA 70%) is reducing 60% the free radicals production when oxidative stress is induced in ARPE-19 cell cultures.[3] Significant differences with the rest of enzymatic triglycerides, from different trademarks, are seen whatever the concentration used.

ANTIOX. EFFECT OF TRIGLYCERIDES WITH VARIABLE DHA CONCENTRATIONS



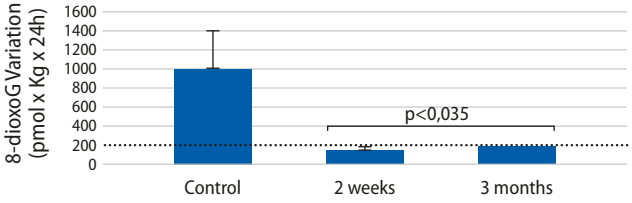
TRIDOCOSAHEXAENOINA-AOX® ANTIOXIDANT EFFECT

Clinical trials

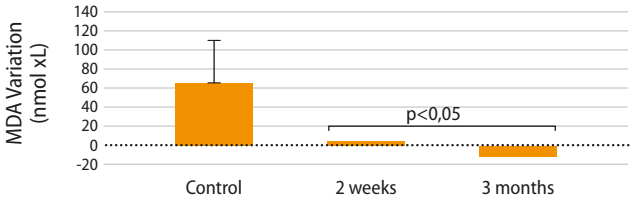
In Sport[4]

Protection against oxidative stress induced with intense physical exercise by means of a 90 minutes effort load in (n=40) healthy sportsmen. Tridocosahexaenoina-AOX® is supplemented during 3 months following a randomized; placebo controlled, and crossed trial design. Significant differences versus the basal levels can be seen after 2 weeks and after 3 months in the DNA oxidation metabolites found in the 24 hours urine collection, and also in the serum lipid peroxidation levels.

OXIDIZED DNA IN 24 HOUR URINE COLLECTION



SERUM LIPID PEROXIDATION

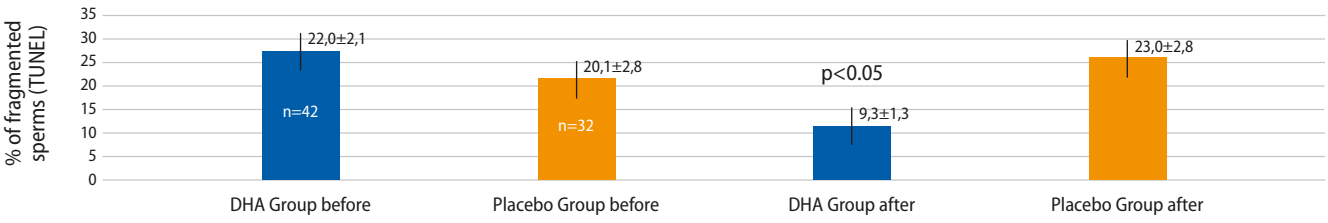


Time supplementing with Tridocosahexaenoina-AOX®

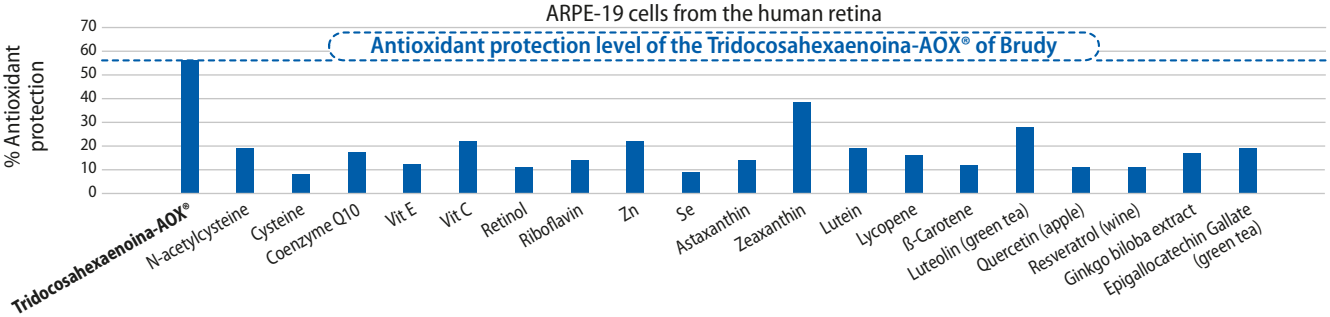
In Fertility[6]

Effects of supplementing Tridocosahexaenoina-AOX® (n=42) (Brudy Plus 3 capsules/day: 1000mg/day) versus Placebo (n=32) during 10 weeks in patients with poor sperms DNA integrity, by means of TUNEL technique with fluorescent DNA markers. Significant differences can be seen in the percentage of oxidized sperms showing less fragmented DNA in the actively supplemented group versus in the Placebo group.

DNA FRAGMENTATION



OXIDATIVE PROTECTION IN ARPE-19 CELL CULTURE
Comparing the antioxidant effect of DHA with other available antioxidants



TRIDOCOSAHEXAENOINA-AOX® ANTI-INFLAMMATORY EFFECT

Glutathione reduces the oxidative harm in the cell, and is the main actor controlling the inflammatory process[3,7-11]

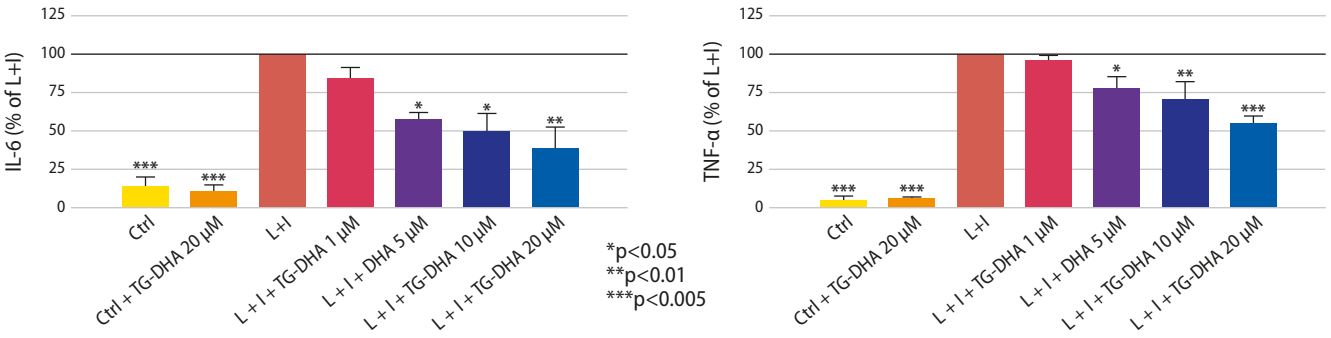
01 Stimulates the synthesis of intracellular glutathione, protector of oxidative damage.[3]

02 It blocks synthesis of pro-inflammatory E2 Eicosanoids, and stimulates synthesis of anti-inflammatory E3 Eicosanoids (need of reducing animal meat and fat consumption).

03 It promotes resolving docosanoids: D1 Protectin plus E1 and D1 Resolvins derived from DHA and EPA, which facilitates resolution of inflammation.

04 It inhibits Nuclear Factor-κβ activation, blocking the synthesis of some pro-inflammatory cytokines as: IL-6, TNF-α and IL-1β.

TRIDOCOSAHEXAENOINA-AOX® "IN VITRO" EFFECT IN A CELL CULTURE[7]

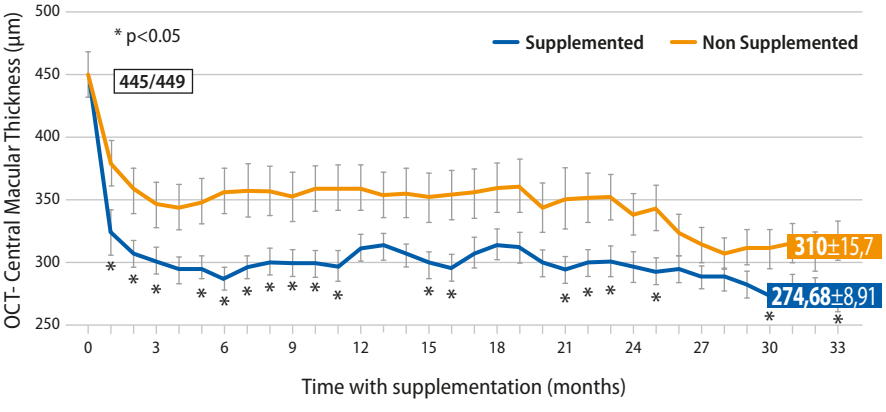


Tridocosahexaenoina-AOX® inhibitory effect on the NF-κβ activation in BV2 microglia activated with bacterial lipopolysaccharide + interferon-Gamma (L+I). It can be seen a dose-dependent, significant reduction on the cytokines synthesis: IL-6 and FNT-α.

Clinical results

Antiinflamatory-Antiedema effect in central macula in (n=61) patients suffering Diabetic Macular Edema, treated all of them with intravitreal Ranibizumab, and 50% randomly assigned either to receive or not Tridocosahexaenoina-AOX® supplementation for 36 months. Sustained significant differences in the central macular thickness (+P<0.05), measured with Optical Coherence Tomography (OCT), in the supplemented group versus the non supplemented group.

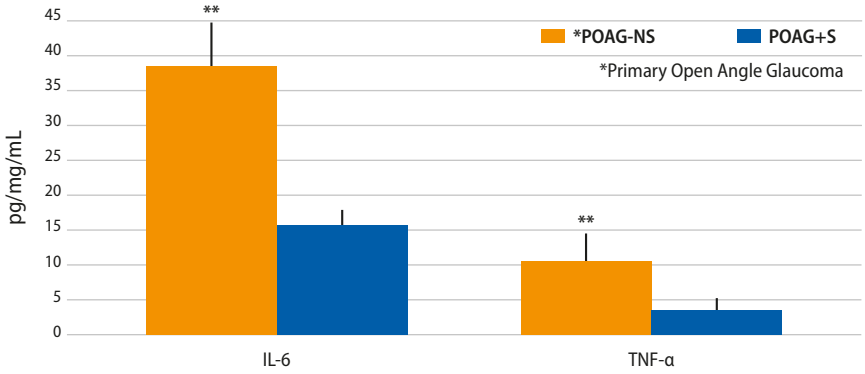
ANTI-INFLAMMATORY EFFECT AT CENTRAL MACULAR LEVEL[11,22]



EXPRESSION OF CYTOKINES IN TEARS AFTER SUPPLEMENTATION[8-10]

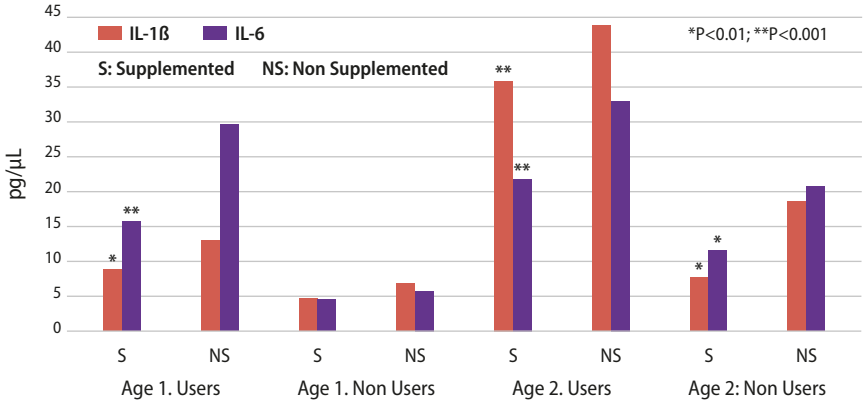
Inhibitory effect on the cytokines produced in the tears in (n=31) patients suffering Primary Open Angle Glaucoma (POAG) being users of topical anti-glaucoma eye-drops. Patients are 50% supplemented or not with Tridocosahexaenoina-AOX® at random during 90 days. Significant lower levels of IL-6 and FNT-α can be seen in the supplemented POAG group of patients versus the non-supplemented POAG group of patients. There is a significant improvement of all the studied clinical variables.

CYTOKINES IN TEARS (Glaucoma)[9]

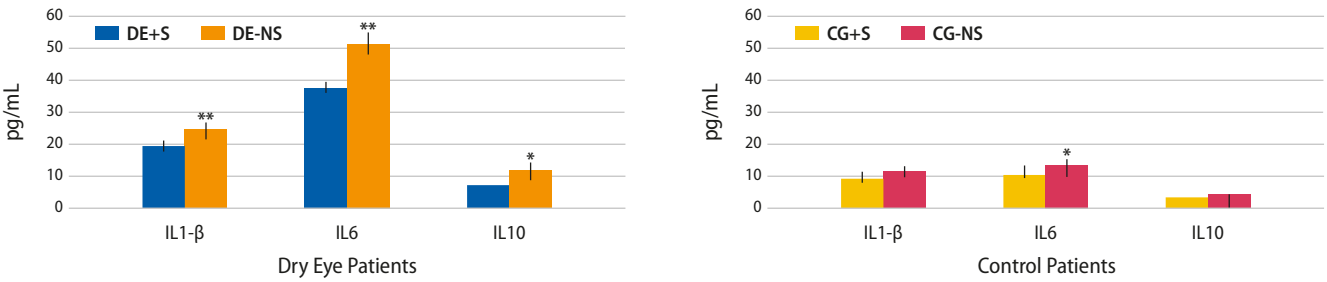


Expression of inflammatory markers (IL-6 and IL-1B) in reflex tears of n=83 women intensive users of computer screens, and in n=65 women non users (Controls). They are analyzed in two different age ranks (Age 1: 40-52 years; Age 2: 53-65 years), 50% randomly assigned either to receive or not supplementation with Tridocosahexaenoina-AOX® during 90 days. *P<0.01; **P<0.001
Active supplementation had a positive influence on the ocular surface pathology, showing evident and significant improvement of the clinical signs and symptoms derived from the abusive use of screen computers.

CYTOKINES IN TEARS (Intensive users of computer screens)[10]



EFFECT OF ORAL SUPPLEMENTATION ON THE CYTOKINES EXPRESSED IN TEARS[8]



Synthesis of Tear cytokines is significantly reduced in (n=30) patients suffering moderate dry eye, and in (n=36) healthy controls, 50% of them being Supplemented or not at random with Tridocosahexaenoina-AOX® during 90 days. A statistical significant lower tear levels of IL-6, IL-1B, and IL-10 are detected in the actively supplemented Dry Eye patients (DE+S) and in the actively supplemented Controls (CG+S) than in the non supplemented Dry Eye patients (DE-NS) and in the non supplemented group of Controls (CG-NS). (*P<0.01, **p<0.001). A significant improvement of all clinical variables are also detected.

EXPRESSION OF CYTOKINES AT SYSTEMIC LEVEL (SERUM) AFTER SUPPLEMENTATION, AND LEVEL OF ANTIOXIDANT PROTECTION[20-22]

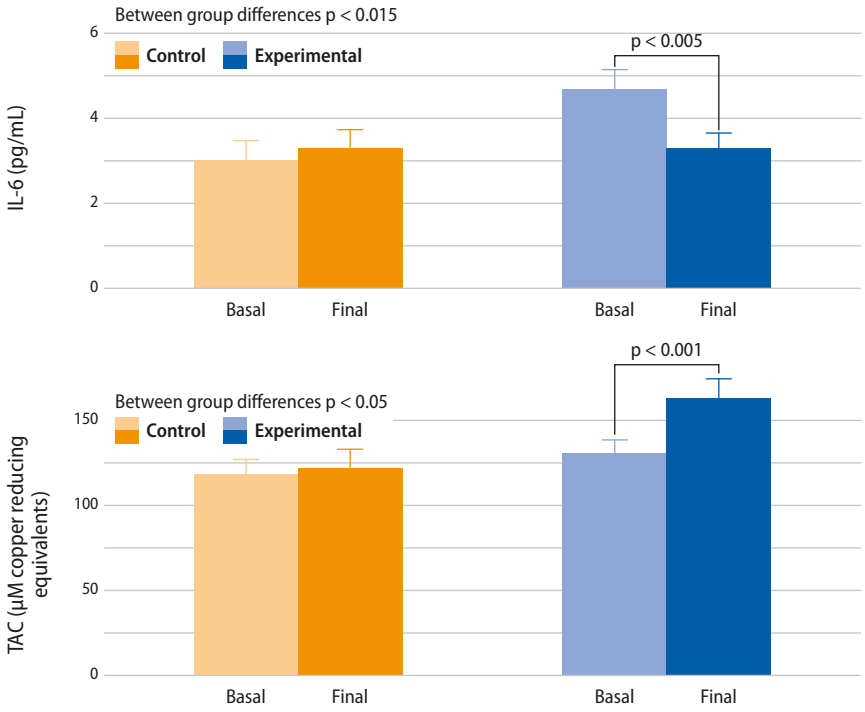
Tridocosahexaenoina-AOX® shows a clear inhibiting effect on IL-6 synthesis at systemic level, but also an increase in the Total Antioxidant Capacity at short, medium and long term, in patients suffering Diabetic Retinopathy and Pseudoexfoliation Glaucoma.

At short term: 90 days[20]

N=24 patients suffering Non Proliferative Diabetic Retinopathy. 50% being supplemented with Tridocosahexaenoina-AOX 1000mg/day (BrudyRetina, 3 capsules/day) during 90 days. Control group not being supplemented.

Significant reduction in the IL-6 blood levels is only seen in the actively supplemented group, also showing between group significant differences. A worsening is seen in the control group. A significant increase of the Total Antioxidant Capacity is only seen in the actively supplemented group[20].

IL-6: Interleukin-6
TAC: Total Antioxidant Capacity

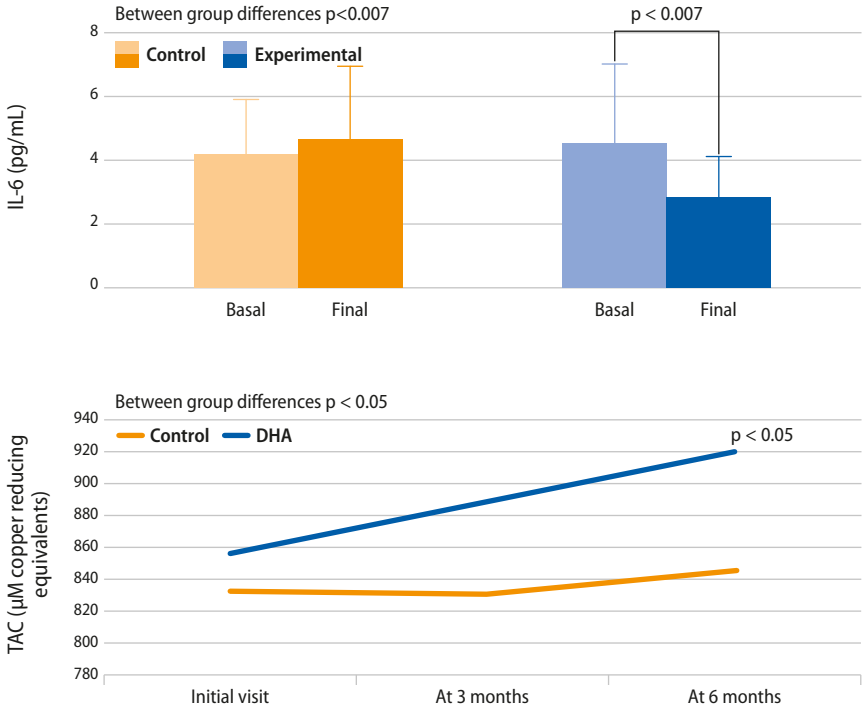


At mid term: 6 months[21]

N=47 patients suffering pseudoexfoliation glaucoma. 50% of the patients are supplemented with tridocosahexaenoina-AOX. 1000mg/day (BrudyPio 3 capsules/day) during 6 months; control group not being supplemented.

Significant reduction in the IL-6 blood levels is only seen in the actively supplemented group, also showing between group significant differences. A worsening is seen in the control group. A significant increase in the Total Antioxidant Capacity is only seen in the actively supplemented group[21].

IL-6: Interleukin-6
TAC: Total Antioxidant Capacity

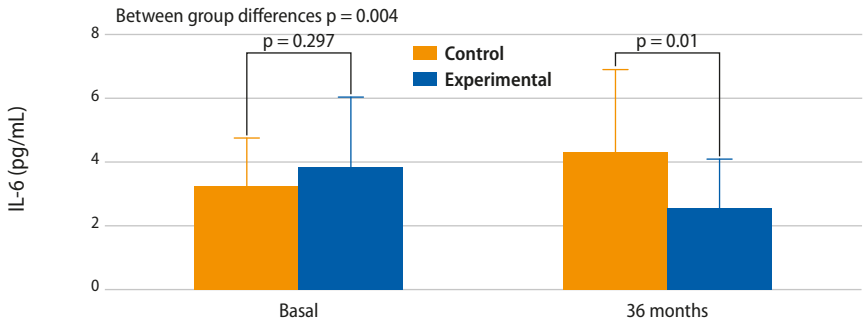


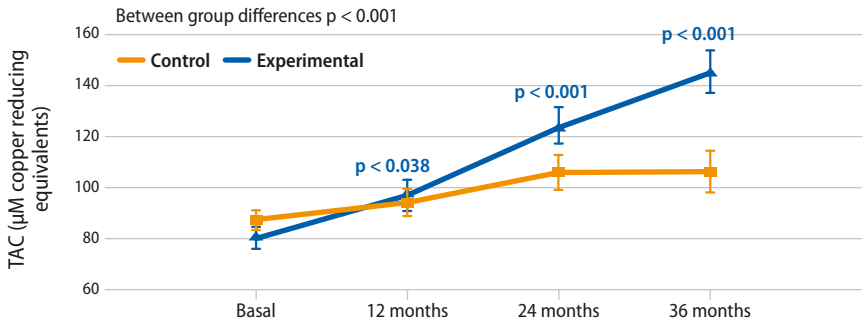
At long term: 3 years[22]

N=60 patients with type 2 diabetes, suffering Diabetic Macular Edema. 50% of patients being supplemented with Tridocosahexaenoina-AOX 1000mg/day (BrudyRetina 3 capsules/day) during 36 months. Control group not being supplemented. All patients are treated with intravitreal injections of Ranibizumab.

Significant reduction in the IL-6 levels is only seen in the actively supplemented group, also showing between group differences. A worsening is seen in the control group. A significant increase in the Total Antioxidant Capacity is only seen in the actively supplemented group[22].

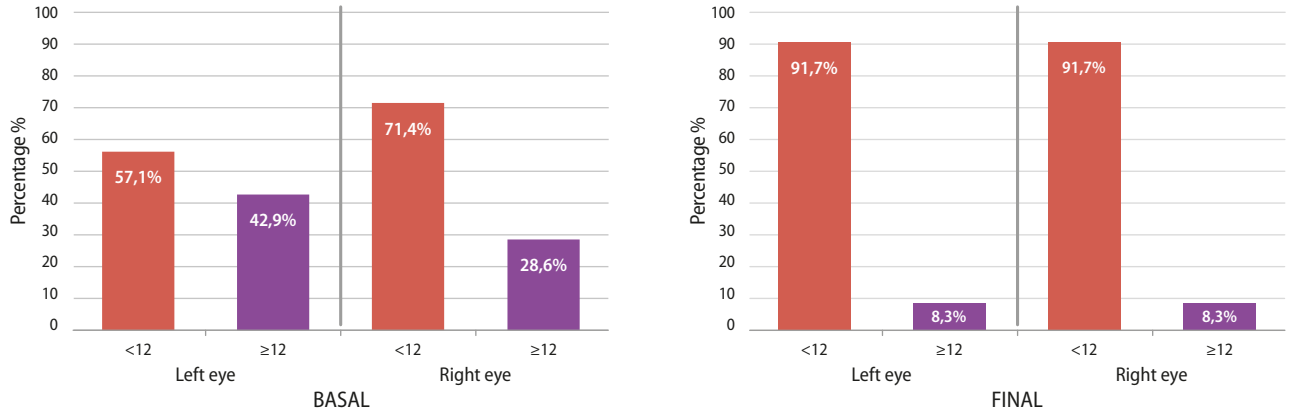
IL-6: Interleukin-6
TAC: Total Antioxidant Capacity





TOPICAL EYELID APPLICATION OF A GEL-CREAM WITH TRIDOCOSAHEXAENOINA-AOX®[25]

CLDEQ-8 QUESTIONNAIRE (Index>12 indicates risk of suffering dry eye)



Risk of suffering dry eye is evaluated in N=30 healthy volunteers wearers of soft contact lenses after repeated night time eyelid application for 2 weeks. Scoring >12 identifies contact lens wearers being in risk. CLDQ = Contact Lens Dry Eye Questionnaire). BrudyDerm Dry Eye Gel-Cream for eyelid application incorporates Tridocosahexaenoina-AOX, Hyaluronic acid, and Aloe Vera leaf juice. Risk of suffering dry eye is reduced in both eyes.

TRIDOCOSAHEXAENOINA-AOX® IN DRY EYE[8,10,12-16]

Clinical Experience

Prospective, controlled, interventional, randomized clinical trials[8,10,12]

TRIAL ARTICLE	TITLE / THEME	MATERIAL & METHODS	RESULTS	P VALUE
Pinazo-Duran, MD, et al; Clinical Int Aging 2013; 8:139-148	Supplementation effect in moderate dry eye and on the inflammatory markers present in reflex tears	30 moderate Dry Eye patients and 36 healthy controls 50% randomized to receive or not 2 caps/day x 90 days	Schirmer B.U.T. OSDI: -64% Cytokines expression: IL-1β, IL-6, IL-10	<0.05 <0.05 <0.01 <0.001
Ribelles A, et al; BioMed Research Int 2015; 467039 Id 467039. doi: 10.1155/Epub 2015 Oct 18	Supplementation effects in women intensive computer screen users and on the inflammatory markers present in reflex tears	Non Users, 50% randomized to receive or not 3 caps/day x 90 days	Schirmer OSDI Tear Volume (µl) Cytokines expression: IL-1β y IL-6	<0.0002 <0.05 +25% <0.001
Galbis-Estrada C, et al; Molecular Vision 2015; 21:555-567	Supplementation effect on Tears metabolomics of mild and moderate Dry Eye	22 patients with mild Dry Eye and 33 patients with moderate Dry, and 33 healthy controls 50% randomized to receive or not 3 caps/day x 90 days	Schirmer: Mild group Moderate group BUT: Mild group Moderate group OSDI Metabolomic profile Dry Eye vs Control G Metabolomic profile Before vs After Supplementation	 <0.034 <0.039 <0.001 <0.000 <0.05 <0.05

Taking a sample from the reflex tears from the inferior lacrimal meniscus with a capillary tube. Measuring cytokines by means of flux cytometry with the Multi-Plex System (Luminex R-200, Human Cytokine/Chemokine Panel)

Prospective, double-blinded, randomized, placebo-controlled trial in Meibomian Gland Dysfunction[13,14]

TRIAL ARTICLE	TITLE / THEME	MATERIAL & METHODS	RESULTS	P VALUE
Oleñik A, et al; Clinical Int Aging 2013; 8:1133-1138	Supplementation effects in Meibomian Gland Dysfunction patients	60 M.G.D. patients, 50% randomized either to active supplementation or to Placebo 3 caps/day x 90 days	Schirmer BUT OSDI Lid margin redness Meibom expression Oxford grading test	<0.01 <0.001 <0.001 <0.01 <0.01 NS
Oleñik A, et al; Clinical Ophthalmol 2014; 8:831-836	Supplementation effects on the quality of life of M.G.D. patients	60 M.G.D. patients, 50% randomized either to active supplementation or to Placebo 3 caps/day x 90 days	Physical component Mental component Placebo	<0.000 <0.0002 NS

Prospective, interventional, open-label[15,16]

TRIAL ARTICLE	TITLE / THEME	MATERIAL & METHODS	RESULTS	P VALUE
Oleñik A, and DECSG; Clinical Ophthalmol 2014:8 169–76	Efficacy and Digestive tolerability in moderat Dry Eye patients	905 Moderate Dry Eye patients supplemented with 3 caps/day x 90 days	Symptoms improvement Average drops/day Satisfyed+Very Satisfyed Large+Very L clin improv 24 h lasting symptoms GI Adverse effects: None Some Fishy taste regurgitation Nausea Vomiting Diarrhea	<0.001 <0.001 82,1% 87,8% -19,4% 80% 20% 13,5% 4,9% 0,3% 1,3%
Gatell-Tortajada J, et LDECSG; Clin Int Aging 2016; 11:571-578	Efficacy and Digestive tolerability in moderat Dry Eye patients	1419 Moderate Dry Eye patients supplemented with 3 caps/day x 90 days	Improv individual sympt Oxford grading test Tear B.U.T. Schirmer Test Hyperemia Conjunctiva Satisfyed+Very Satisfyed Large+Very L clin improv GI Adverse effects: None Some Fishy taste regurgitation Nausea Diarrhea Vomiting	<0.001 <0.001 <0.001 <0.001 <0.001 85,7% 91,6% 79,2% 20,8% 14,6% 4,6% 2,7% 0,4%

TRIDOCOSAHEXAENOINA-AOX® IN DIABETIC RETINOPATHY[11,20,22]

Clinical Experience

TRIAL ARTICLE	TITLE / THEME	MATERIAL & METHODS	RESULTS	P VALUE
Elena Rodríguez González-Herrero, et al; Communication presented at the XXI Congress of the Spanish Society of Retina and Vitreous, Madrid, Mars 3, 2017. Sent to Clinical Ophthalmology 2018	Supplementation with DHA in Non Proliferative Diabetic Retinopathy. Prospective, interventional and controlled trial of the macular function by means of mycroperimetry	N=24 patients suffering NPDR. %0% of them being supplemented (Tridocosahexaenoina-AOX 1000mg/day, Brudyretina 3 caps/day) during 3 months; Control group not being supplemented	VA Changes Macular sensitivity Macular Integrity Index Macular Thickness (OCT) Total Antioxidant Capacity DHA in RBC membrane NEI-VFQ25 IL-6 expression in serum	NS <0.05 <0.05 NS <0.001 <0.05 <0,04 <0.005
Pinazo-Duran MD, et al; *Publication is pending*	Effects on the Macular Pigment Density by supplemmenting lutein/Zeaxanthin, with and without Tridocosahexaenoina-AOX®	N=30 healthy volunteers supplemented with lutein, 50% with DHA-TG and other 50% without it	Optical Density of Macular Pigment	*Trial is ongoing; sample of participants has been increased*
Lafuente M, et al; Journal of Retina 2017; 37:1277-1286	Effects of supplementation in patients suffering Diabetic Macular Edema treated with intravitreal Ranibizumab	62 patients (76 eyes) randomly assigned to supplementation or not with 3 caps/day x 24 months	OCT intragroup: With supplement Without supplement OCT intergroup EDTRS-VA intergroup >5 Letters DHA group >10 Letters DHA gr DHA in erythrocyte membrane ω6/ω3 index erythrocyte Total Antiox Capacity	 <0.001 <0.024 <0.05 <0.066 <0.044 <0.044 <0.01 <0.05 <0.001
Lafuente M, et al; Communication presented at the XXI Congress of the Spanish Society of Retina and Vitreous, Madrid, Mars 4, 2017. RETINA 2018; 2018 Feb 22. doi: 10.1097/IAE.0000000000002114. [Epub ahead of print]	Tridocosahexaenoina-AOX® supplementation effects on Diabetic Macular Edema suffering patients treated with intravitreal Ranibizumab. Results at 36 months	N=60 patients (74 eyes) treated with iv Ranibizumab; 50% of patients additionally supplemented with DHA-TG 1000mg/day (BrudyRetina: 3 capsules/day) during 36 months	Central Macular Thickness BCVA Metabolic Control (HbA1c) Total Antioxidant Capacity DHA in RBC membrane IL-6 expression in serum	0,035 NS 0.035 0.001 0.001 0.004

TRIDOCOSAHEXAENOINA-AOX® IN GLAUCOMA[9,17,21]

Clinical Experience

TRIAL ARTICLE	TITLE / THEME	MATERIAL & METHODS	RESULTS	P VALUE
Galbis-Estrada C, et al; Clinical Int Aging 2013; 8:711-719	Effect of supplementation on Dry Eye due to Glaucoma and on inflammatory markers in tears	31 Glaucoma+Dry Eye patients, 31 healthy controls, and 30 Dry Eye patients with no Glaucoma, 50% supplemented at random with 2 caps/day x 90 days	Schirmer Test Tear BUT OSDI (-68%) Rose Bengal staining VA Cytokines in tears: IL-6 TNF-α	<0.002 <0.02 <0.05 NS NS <0.05 <0.001
Tellez-Vázquez J, and the DEIGSG; Clin Ophthalmol 2015; 10: 617-626	Effect of supplementation in a large series of patients with Dry Eye suffering POAG	1255 POAG+Dry Eye patients Gbeing supplemented with 3 caps/day x 90 days	Symptoms Hyperemia conjuntiva Oxford grading Test Tear BUT Schirmer Test IOP Average tear drops Satisfyed+Very Satisfyed Large+Very L clin improv GI Adverse effects: None Some Fishy taste regurgitation Nausea Diarrhea Vomiting	<0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 81,9% 87,7% 76,5% 23,5% 16,9% 4,7% 1,0% 0,3%
Ongoing Trial	Supplementation of Tridocosahexaenoina-AOX® alone versus Vitamins, versus Citicoline, versus Tridocosahexaenoina-AOX®+Citicoline together	N=80 patients diagnosed of primary Glaucoma, randomized to each one of the 4 arms of supplementation	*Trial is still in process*	
Stéphanie Romeo, et al; Communication at the European Glaucoma Society Congress 2018 in Florence; Poster Abstract ID: 2086; sent for publication to Journal of Glaucoma 2018	Influences of DHA-TG Supplementation in Exfoliation Glaucoma	N=47 patients suffering Exfoliation Glaucoma being 50% supplemented with Tridocosahexaenoin-AOX 1000mg/day (BrudyPio 3 caps/fday) during 6 months; Control group is not supplemented	BCVA Papillary diameters IOP Right Eye IOP Left Eye OCT thickness DHA in RBC membrane membrane n-6/n-3 Index Total Antioxidant Capacity IL-6 expression in serum	NS NS <0.02 <0.007 NS <0.0001 <0.0005 <0.02 <0.006

TRIDOCOSAHEXAENOINA-AOX® IN THE EYELID AREA[25]

Clinical Experience

TRIAL ARTICLE	TITLE / THEME	MATERIAL & METHODS	RESULTS	P VALUE
Zanón-Moreno V et al; sent for publication to J of Optometry	Effects on the eyelid skin integrity and on the ocular surface of a repeated night-time application of a Gel-cream containing Tridocosahexaenoina-AOX®	N=60 healthy volunteers, n=30 contact lens users and n=30 non contact lens users with one night-time application during 2 weeks	Evident CLDEQ-8 Improvement There is OSDI improvement Schirmer test improvement TBUT Improvement Right Eye TBUT Improvement Left Eye VEGF expression in tears Cosmetic improvement	NS NS NS =0.008 =0.005 NS 54-86%

TRIDOCOSAHEXAENOINA-AOX® IN FERTILITY[6,19,24]

Clinical Experience

TRIAL ARTICLE	TITLE / THEME	MATERIAL & METHODS	RESULTS	P VALUE
Martinez-Soto JC, et al; Fertility & Sterility 2010; 94:S235-S236	Supplementation influence in Sperm DNA integrity (DNA oxidative fragmentation)	46 patients 50% supplemented at random either to active or placebo 1g/day x 10 weeks	% reduction of DNA sperms fragmentation Improved seminal fluid Antioxidant capacity	<0.01 <0.01
Popova A Yu, et al; Andrology & Genital Surgery 2015; 16(2): 51-55	Effect of supplementation on the % of sperms DNA fragmentation >15%	40 Pathozoospermic patients, 20 with Tridocosahexaenoina-AOX® and 20 with antiox vitamins and minerals x 45 days	Reduction in the % of fragmented sperms (TUNEL) Active supplemented group Basal 25,8% Control group basal 25,3%	13,7% <0.05 19,8%
Martinez-Soto JC, et al; Syst Biol Reproductive Med 2016; 62(6):387-395	Improved seminal fluid antiox cap. and sperm DNA fragmentation. Double blind, placebo-controlled	74 males randomized to: 32 with Placebo 42 with Active 1g/day x 10 weeks	DNA Sperms fragmentation analyzed with TUNEL technique Seminal fluid antiox capacity DHA levels in seminal fluid	<0.01 <0.01 <0.01

TRIDOCOSAHEXAENOINA-AOX® IN SPORT[4,18]

Clinical Experience

TRIAL ARTICLE	TITLE / THEME	MATERIAL & METHODS	RESULTS	P VALUE
Guzman JF, et al; Journal of Sports Sci Med 2011;10:301-5	Tridocosahexaenoina-AOX® improves complex reaction time in elite women soccer players	24 elite women soccer players 50% randomized to Active or Placebo 3,5g/day x 4 weeks	Complex reaction time Precision efficiency Improvement in neuromotor function is shown	<0.004 <0.003
Contreras CJ, Tesis Doctoral 2014, Universidad Católica Murcia	Supplementation effect on the oxidative protection of intense exercise	40 young healthy cyclist with a controlled efford load; 2g x 3 months	DNA oxidative metabolites in urine at 2 weeks / 3 months: Lipid Peroxidation at 2 weeks / 3 months:	<0.05 <0,05
Antonio J Luque, et al; *Pending Publication*	Tridocosahexaenoina-AOX® versus Placebo in the inflammatory response and muscular harm in Triathletes	N=35 Triathletes randomized to Active or Placebo x 12 weeks, wash-up priod of 30 days and crossing supplements for an other 12 weeks period	*Analysing statistical results*	

TRIDOCOSAHEXAENOINA-AOX® IN EXPERIMENTAL RESEARCH[3,7]

Clinical Experience

TRIAL ARTICLE	TITLE / THEME	MATERIAL & METHODS	RESULTS	P VALUE
Bogdanov P, et al; IOVS ARVO Journals, May 2008, Vol 49, 5932	The Influence of Tridocosahexaenoina -AOX® in oxidative protection of human retinal pigmentary epithelium cells (ARPE-19)	Influence of DHA-TG on the intracellular process of Glutathione synthesis	Supplementation is significantly stimula-ting glutathione production in the cytoplasm, protecting the DNA of cells	<0.05
Mancera P, et al; Nutrients 2017; 9:681, doi: 10.3390/nu9070681	The influence of Tridocosahexaenoina -AOX® in the microglia activation and on the improvement of the induced encephalo-mielitis in mice	Incubation of BV-2 microglia in DHA-TG is significantly reducing cytokines synthesis in a concentration dependent manner. Is also significantly reducing the clinical symptoms of encephalomielitis comparing with placebo	<u>BV-2 Cells:</u> IL-6 expression Expression of TNF-α Nitrites levels <u>Induced encephalo-mielitis in mice:</u> Clinical Scoring Weight gain	 <0.05-0.001 <0.05-0.005 <0.005 <0.05 <0.01

TRIDOCOSAHEXAENOINA-AOX® IN DISLIPEMIAS[5]

Clinical Experience

TRIAL ARTICLE	TITLE / THEME	MATERIAL & METHODS	RESULTS	P VALUE
Domingo P; Clinical Nutrition 2017; http://dx.doi.org/10.1016/j.clnu.2017.05.032	Supplementation of Tridocosahexaenoina-AOX® versus Placebo in HIV active patients suffering dislipemia. Randomized, double-blinded, placebo controlled trial	N=84 patients HIV active randomized to either supplementation with DHA-TG 4,9g/day or Placebo during 48 weeks	Reduction in triglyceridemia: Week 4 (44% vs 3%): Week 12 (44% vs 3%): Week 48: Week 4 correlated with DHA in the RBC membrane:	 <0.0001 <0.05 <0.03 r =0.7110, < 0.0001

TRIDOCOSAHEXAENOINA-AOX® IN OTHER FIELDS OF HEALTH[23]

Clinical Experience

TRIAL ARTICLE	TITLE / THEME	MATERIAL & METHODS	RESULTS	P VALUE
Pending Publication	Tridocosahexaenoina-AOX® versus Placebo in Amyothrophic Lateral Sclerosis	N=28 patients affected of ELA randomized either to Active 4,9 g/day or to Placebo x 36 months	*This trial is still in process of execution*	
Pending Publication	Efecto de la Tridocosahexaenoina-AOX® versus Placebo in Chronic Lymphatic Leukemia	Double-Blind, Placebo-controlled. At long term	*This trial is still in process of execution*	
Rodríguez C, et al; *to be sent for publication*	influence of supplementing Tridocosahexaenoina-AOX® versus Placebo in Attention Deficit Hyperactivity Disorder in children	N=98 children suffering ADHD under Methylphenidate treatment or not, being 50% supplemented either with Tridocosahexaenoina-AOX® (1 or 2g/day of BRUDYNEN deppending on body weight), or with placebo during 6 months	*Trial is finished and extracting statistical conclusions*	

Own clinical experience: Tridocosahexaenoina-AOX® published trials

1. Reglamento CE Nº 1881/2006 (19 diciembre 2006) que fija el contenido máximo de contaminantes en alimentos y modificación Nº 1259/2011.

2. Linda M Arterburn, et al; Distribution, interconversion, and dose response of ω-3 fatty acids in humans; Am J Clin Nutr 2006;83(suppl):1467S–76S.

3. Bogdanov P, et al; Docosahexaenoic acid improves endogen antioxidant defence in ARPE-19 cells; ARVO Congress 2008, Poster5932/A306, Thursday, May 01, 2008.

4. Carlos J Contreras; Modificación del daño oxidativo en un grupo de ciclistas tras consumir ácido docosahexaenoico a distintas dosis; Tesis Doctoral, Universidad Católica de Murcia, 2014.

5. Pere Domingo, et al; Effects of docosahexanoic acid on metabolic and fat parameters in HIV-infected patients on cART: A randomized, double-blind, placebo-controlled study; Clinical Nutrition 2017; Jun 8. pii: S0261-5614(17) 30214-5. doi: 10.1016/j.clnu.2017.05.032. [Epub ahead of print].

6. Juan Carlos Martinez-Soto; Dietary supplementation with docosahexaenoic acid (DHA) improves seminal antioxidant status and decreases sperm DNA fragmentation; Syst Biol Reproductive Med 2016, 62 (6): 387–395; http://dx.doi.org/10.1080/19396368.2016.1246623.

7. Pilar Mancera, et al; Natural Docosahexaenoic Acid in the Triglyceride Form Attenuates In Vitro Microglial Activation and Ameliorates Autoimmune Encephalomyelitis in Mice; Nutrients 2017, 9, 681; doi:10.3390/nu9070681.

8. M Dolores Pinazo-Durán; Effects of a nutraceutical formulation based on the combination of antioxidants and ω-3 essential fatty acids in the expression of inflammation and immune response mediators in tears from patients with dry eye disorders; Clinical Int Aging 2013; 8:139-148.

9. C. Galbis-Estrada; Patients undergoing long-term treatment with antihypertensive eye drops responded positively with respect to their ocular surface disorder to oral supplement with antioxidants and essential fatty acids; Clin Int Aging 2013;8:711-9.

10. Ribelles Alfredo, et al; Ocular Surface and Tear Film Changes in Older Women Working with Computers; BioMed Research International 2015; Article ID 467039.

11. Maria Lafuente, et al; Combined intravitreal ranibizumab and oral supplementation with docosahexaenoic acid (DHA) and antioxidants for Diabetic Macular Edema: 2-year randomized single-blind controlled trial results; Published in Retina 2017; 37:1277-1286.

12. Carmen Galbis Estrada, et al; A metabolomic approach to dry eye disorders. The role of oral supplements with antioxidants and omega 3 fatty acids; Molecular Vision 2015; 21:555-567.

13. Andrea Oleñik, et al; A randomized, double-masked study to evaluate the effect of omega-3 fatty acids supplementation in meibomian gland dysfunction; Clinical Int Aging 2013; 8:1133-1138.

14. Andrea Oleñik, et al; Benefits of Omega-3 fatty acid dietary supplementation on health-related quality of life in patients with Meibomian Gland Disfunction ;Clinical Ophthalmol 2014; 8:831-836.

15. Andrea Oleñik, et al; Effectiveness and tolerability of dietary supplementation with a combination of omega-3 polyunsaturated fatty acids and antioxidants in the treatment of dry eye symptoms: results of a prospective study; Clinical Ophthalmology 2014;8:831-6.

16. Jordi Gatell-Tortajada, et al; Oral supplementation with a nutraceutical formulation containing omega-3 fatty acids, vitamins, minerals, and antioxidants in a large series of patients with dry eye symptoms: results of a prospective study; Clin Int Aging 2016; 11:571-578.

17. Jesús Tellez-Vazquez, et al; Omega-3 fatty acid supplementation improves dry eye symptoms in patients with glaucoma: results of a prospective multicenter study; Clin Ophthalmol 2016; 10:617-626.

18. Guzman JF, et al; DHA- rich fish oil improves complex reaction time in female elite soccer players ;Journal of Sports Sci Med 2011;10:301-5.

19. Popova A Yu, et al, Experience in the use of docosahexaenoic acid (BrudyPlus) in patients with increased spenn DNA fragmentation index in Acad. V.I. Kulakov Research Center for Obstetrics, Gynecology and Perinatology; Andrology & Genital Surgery 2015; 16(2): 51-55.

20. Elena Rodriguez, et al; Supplementation with docosahexaenoic acid and antioxidants in nonproliferative diabetic retinopathy: prospective controlled study of macular function by fundus microperimetry; sent to Clinical Ophthalmology 2018 (ID 157635). Communication in the 21st Congress of the Spanish Society of Vitreous and Retina; Madrid, Mars 3, 2017.

21. Stéphanie Romeo, et al; Antioxidant and Anti-inflammatory Effects of Docosahexaenoic Acid (DHA) Plus Vitamins and Minerals Supplementation in Pseudoexfoliative Glaucoma: A 6-Month Open-label Randomized Trial; Sent for publication to Journal of Glaucoma 2018. Poster Abstract ID: 2086 presented at the 13 European Glaucoma Society Congress, 20 May, Florence (Italy).

22. María Lafuente, et al; Three-year outcomes in a randomized single-blind controlled trial of intravitreal ranibizumab and oral supplementation with docosahexaenoic acid and antioxidants for diabetic macular edema. RETINA 2018; 2018 Feb 22. doi: 10.1097/IAE.0000000000002114. [Epub ahead of print].

23. Rodríguez C, et al; Efectos del Triglicérido de DHA en pacientes con trastornos por déficit de atención con o sin hperactividad; estudio clínico randomizado, a doble ciego, controlado con placebo. Estudio finalizado en 2017 y analizando los resultados; Publicación pendiente.

24. Juan Carlos Martinez-Soto, et al; Effect of dietary DHA supplementation on sperm DNA integrity; Fertility & Sterility 2010; 94(4):S235-S236.

25. Zanón-Moreno V, Raga-Cervera J, Sanz-González S, Pinazo-Durán MD, Effects of the nocturnal application of a dermatologic gel-cream containing docosahexaenoic acid and hyaluronic acid on the palpebral and ocular surface integrity. Enviado a J. Optometry 2018.

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